

The listing of claims presented below replaces all prior versions and listings of claims in the application.

CLAIMS

1. (Currently Amended) Circular recombinant plasmid DNA construct encoding a) a protein tag, b) a visual marker protein, and containing c) a multiple cloning site suitable for insertion of an additional target protein gene arranged in series in the above order ~~gene~~, characterised in that it further contains d) a frame adaptor of variable length between the visual marker and protein tag ~~gene genes and the visual marker protein genes in that the gene sequence encoding~~ the protein tag, the visual marker protein gene and the frame adaptor are being specifically ~~designed and~~ engineered at the DNA level for respectively i) immobilisation purposes at the protein level wherein metal ion is required to enable the immobilisation event, ii) visualisation and quantification purposes at the protein level and, iii) providing a large distance separating protein and surface to enable the ~~immobilised~~ immobilized enzymes to display native-like characteristics.
2. (Currently Amended) Construct according to claim 1, characterised in that the protein tag is chosen from the group containing lysine (lys), histidine (his), tyrosine (tyr), phenylalanine (phe), arginine (arg), glutamic acid (glu), aspartic acid (asp), glutamate, aspartate, asparagine (asn), glycine (gly), glutamine (gln), alanine (ala), valine (val), and tryptophan (trp).
3. (Previously Presented) Construct according to claim 1, characterised in that the protein tag is a

histidine-tag such as a polyhistidine variant, in particular (6X) histidine.

4. (Previously Presented) Construct according to claim 1, characterised in that the visual marker protein is chosen from the group containing fluorescent or phosphorescent proteins, wherein the fluorescent protein is chosen from the group containing Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), Yellow Fluorescent Protein (YFP) and Blue Fluorescent Protein (BFP) as well as their variants and/or mutants.

5. (Currently Amended) Construct according to claim 1, characterised in that the multiple cloning site contains restriction enzyme recognition sites chosen from the group containing SacI, Sal I, Hind III, Eag I, and Not I.

6. (Previously Presented) Construct according to claim 1, characterised in that it expresses a fusion protein, wherein the tag is suitable to interact directly with appropriate surface pendant groups of a support material.

7. (Previously Presented) Construct according to claim 6, characterised in that the direct interaction with the support material is covalent or non-covalent.

8. (Previously Presented) Construct according to claim 7, characterised in that the direct interaction is non-covalent and yet freely accessible and leach-free like covalent one.

9. (Withdrawn) Method for preparing and immobilising a protein on a support material,

comprising:

- a) Engineering at the DNA level, in series a protein tag suitable to interact directly with appropriate surface pendant groups of a support material, a fluorescent marker protein for visualisation and quantification purposes at the protein level and a multiple cloning site suitable for insertion of a target protein to be immobilised,
- b) Inserting the corresponding gene of the target protein to be immobilised into the multiple cloning site;
- c) Initiating protein expression.
- d) Optionally pre-treating the support material;
- e) Incubating the protein and support material together, wherein the protein is immobilised to the support via specific tag-surface interactions;
- f) Washing away the non-specific biomolecules;
- g) Optionally quantifying the fluorescence of the visual marker protein;
- h) Optionally desorbing the target protein;

characterised in that the support material is chosen from the group containing polymers, biopolymers, glass and composites containing silicone dioxides, metals and metal oxides, as well as any combination thereof on the microscopic, mesoscopic or macroscopic length scale.

10. (Withdrawn) Method according to claim 9, characterised in that the support material is chosen from the group containing polymers, silicon dioxides, aluminum oxides, titanium oxides, magnesium oxides, borates, metals and other metal oxides.

11. (Withdrawn) Method according to any one of claims 9 and 11, characterised in that the polymers are chosen from the group containing polyolefins such as polystyrene, polyacrylates, polymethacrylates, polybutylene, polyvinylalcohol and related derivatives, polyvinylchlorides, polyisoprene, polypropylene, polyphenols, polyamides, polyesters polysulfones, polyethersulfones, polyethersulfides, polyimines, polyethyleneglycols, polypropyleneglycols, polyimides, polycarbonates, polyurethanes.

12. (Withdrawn) Method according to claim 11, characterised in that the polymer surface is chemically treated to bear various functional groups chosen between carboxyl groups, hydroxyl groups, amino groups, amide groups, ester groups, imide groups, imine groups, mercapto groups, nitro groups, sulfonate groups, phosphate groups, phosphonate groups, cyano groups, sulfone groups, aldehyde groups, epoxide groups, urethane groups, ketone groups, phenolic groups, aromatic groups, alkyl, alkenyl, alkynyl, acyl and aryl groups, silanol groups, silicon oxide groups, siloxane groups, metal hydroxide groups, metal oxide groups, and elemental metals.

13. (Withdrawn) Method according to claim 9, characterised in that the support material is carboxylated polystyrene.

14. (Withdrawn) Method according to claim 9, characterised in that quantifying in step g) of the fluorescence of the visual marker protein is used in applications selected from analysis, diagnosis (like in enzyme based diagnostic kits), incubation, storage, sensing, arraying and orienting, catalysis, stabilisation, binding, signal transduction, chemical transformation, implant passivation and surface biocompatibilization, surface activation, purification, detoxification and

scavenging.

15. (Withdrawn) A two-component system obtained by claim 9.

16. (Withdrawn - Currently Amended) Circular recombinant plasmid DNA construct encoding a) a protein tag, b) a visual marker protein, and containing c) a multiple cloning site suitable for insertion of an additional target protein gene arranged in series in the above order gene, characterised in that it further contains d) a frame adaptor of variable length between the visual marker and protein tag gene genes and the visual marker protein genes in that the gene sequence encoding the protein tag, the visual marker protein gene and the frame adaptor are being specifically ~~designed~~ and engineered at the DNA level for respectively i) immobilisation purposes at the protein level wherein metal ion is required to enable the immobilisation event, ii) visualisation and quantification purposes at the protein level and, iii) providing a large distance separating protein and surface to enable the ~~immobilised~~ immobilized enzymes to display native-like characteristics.